Research Article

β-Galactosidase optimization using response surface methodology

Mudiganti Ram Krishna Rao*, S. AnandaNarasimhan

ABSTRACT

Objective: β-galactosidase (β-D-galactoside galactohydrolase; E. C. 3.2.1.23), or lactase, catalyzes the breakdown of lactose into glucose and galactose. They are naturally available and are produced by many microorganisms such as bacteria, fungi, and yeast. The present study is to explore the possibility of production of this enzyme by *Lactobacilli fermentum.* **Materials and Methods:** A loop of inoculum was used for inoculating the culture medium and was incubated at pH 4 and temperature 30°C at 125 rpm. The enzyme activity was checked for at 540 nm in DNS assay, and then, the optimized enzyme was partially purified by ammonium sulfate precipitation. The concentrated enzyme was dialyzed and purified and the molecular weight of the enzyme was measured using high-performance liquid chromatography (HPLC). Response surface methodology showed good significance in the optimization of culture medium parameters. **Results:** In the present study, it was found that the extracellular β-galactosidase production by the *L. fermentum* in a submerged skim milk broth was maximum 7.99 U/mL/min enzyme units under optimized the culture medium of its carbon and nitrogen source. **Conclusion:** This method could be exploited for commercial production of beta-galactosidase enzyme.

KEY WORDS: β-galactosidase, *Lactobacillus fermentumin*, HPLC, glucose, galactose, enzyme

INTRODUCTION

Lactose intolerance is prevalent in half the world's population[1] due to the deficiency of the β-galactosidase enzyme. β-galactosidase (E. C 3.2.1.23) is an enzyme of great value in the dairy industry.[2-4] The symptoms of this deficiency include flatulence, bloating, diarrhea, and abdominal pain. The absence of β-galactosidase could be congenital, primary, or secondary deficiency.

Lactic acid bacteria were known to synthesis β-galactosidase for the hydrolysis of lactose and to use them for their growth and these bacteria produce β-galactosidase inside their cell as it is an intracellular enzyme. It is also known to produce, in trace amounts, extracellular β-galactosidase. In the present work, we have attempted to optimize the extracellular β-galactosidase production by *Lactobacilli fermentum* strain extracted from curd and cultured in skim milk broth.

Access this article online	
Website: jprsolutions.info	ISSN: 0975-7619

Siddique *et al.*[5] studied the production of β-galactosidase by solid-state fermentation using wheat bran as a substrate. *Aspergillus niger* and its deoxyglucose-resistant mutant were grown with initial pH of 5.5 and 30°C for 144 h in culture media and samples were harvested after every 24 h to analyze for substrate consumption, cell mass formation, and enzyme production. All the five nitrogen sources used such as ammonium sulfate, corn steep liquor, diammonium phosphate, fish meal, and urea indicated significant results. Higher values of enzyme activity of 168.0 and 371.15 μ/L/h in parent and mutant variety, respectively, were obtained from samples using corn steep liquor as nitrogen sources as compared to control (73.1 and 176.3 u/l/h in parent and mutant, respectively).

In the present study, the production of extracellular β-galactosidase from *L. fermentum* strain extracted from curd. The study also included measuring the level of activity of enzyme by varying the optimal level using RM analysis and to measure the molecular weight of the enzyme using high-performance liquid chromatography (HPLC) technique.

Department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

***Corresponding author:** Dr. Mudiganti Ram Krishna Rao, Department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Chennai - 600 073, Tamil Nadu, India. Phone: +91-9894994567. E-mail: mrkrao1455@gmail.com

Received on: 11-11-2018; Revised on: 16-12-2018; Accepted on: 09-02-2019

MATERIALS AND METHODS

A strain of *L. fermentum* was isolated from homemade curd by an enrichment culture method in APT7302 agar on Petri plates and maintained at 4°C. The inoculums are subcultured in skim milk broth. 100 µL of the suspension containing 10–30 CFU/ml was used as the seed inoculums. 2.8 g of skim milk, 0.5 g of casein enzyme hydrolysate, 0.2 g yeast extract, and dextrose in 250 mL were added with 50 mL distilled water in Erlenmeyer flasks and sterilized. After cooling, a loop containing *L. fermentum* was streaked from the incubated inoculum on APT agar plate and mixed thoroughly. Incubation was conducted at 30°C for 48 h in 125 rpm in 50 ml of seed medium. The activity of the enzyme is measured using hydrolysis of lactose using DNS method and one unit of glucose released was measured as equivalent to one unit of β-galactosidase that hydrolyzes lactose.

To know the concentration of enzyme, Folin–Ciocalteu reaction was used. The blue color was measured at 660 nm in a Shimadzu UV-VIS 2401 spectrometer and protein concentration is measured by Lowry's method.

β-galactosidase obtained was partially purified by ammonium sulfate purification method. The crude enzyme collected after homogenization and to it ammonium sulfate (0–80%) saturation was added and equilibrated for 1 h at 4°. The above mixture precipitated in concentrated form after spinning it for 10 min and high speed. The resultant precipitate containing the concentrated enzymes was suspended in 20 mM acetate buffer at pH 4 and analyzed for β-galactosidase activity. The precipitate of the enzyme formed by ammonium sulfate precipitation was dialyzed extensively against 20 mM acetate buffer, pH 4 using dialysis membrane (HiMedia LA398) overnight at 4°C and was repeatedly changed for new buffer to optimize the purification process. The resulting compound composes of pure enzyme concentration which was further assayed. Response surface method (RSM) was used to optimize lipase production. This process involves three important steps: performing the statistically designed experiments, estimating the coefficients in a mathematical model, and predicting the response and checking the adequacy of the model.

For purification of β-galactosidase, to determining its molecular weight, analytical Shimadzu C-18 column composed of JASCO MD-2013Atvp pump and PDA-10Avp detector was used. The used mobile phase was 80% acetonitrile in water with running time of 15 min. The absorbance was measured at 220 nm. About 100 µg of purified β-galactosidase was fed for HPLC analysis after the peaks of the molecular markers were analyzed. Run time was varied for each

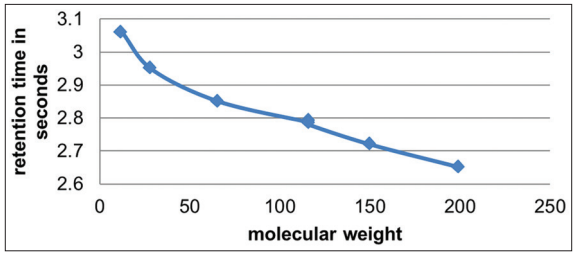

Figure1: The graph between the retention time and the absorbance for B-galactosidase production

run based on the specifications. Moreover, for each marker, their specific molecular weight determines the retention time of the various peaks.

RESULTS AND DISCUSSION

β-galactosidase enzyme was produced under response surface methodology by varying three factors fructose which is the carbon source, casein, and yeast extract which is the nitrogen source and the three factors were cumulatively optimized using RSM. Moreover, the significance of the compounds is shown as three-dimensional graph model, Figure 1. Moreover, the activity level of β-galactosidase has been significantly improved. Moreover, the molecular weight of the purified enzyme was measured using HPLC at retention time of 2.8 s to give a molecular weight of 116 kDa.

CONCLUSION

The crude enzyme and immobilized enzymes were observed to have maximum activity at an acid pH of 4.0 at 30°C. The enzyme was stable in acidic pH range and a temperature range of 30–60°C. The enzyme was stable against various carbon sources and also showed increase in activity in the presence of fructose. These features are desirable for this enzyme preparation to be used in various food processing and pharmaceutical applications.

Partial purification of the enzyme by ammonium sulfate and dialysis was done which resulted in a yield of 43.5% at 0–80% concentration ammonium sulfate and also made to run under high-performance liquid chromatography and had resulted to a peak formation under a retention time of 2.76 s.

The application of enzyme was studied using hydrolysis of lactose. The hydrolysis of lactose finds major role in food processing industry (to improve the texture and sweetness of dairy production) and pharmaceutical industry (to treat lactose intolerant people). The optimum condition for the enzyme from *L. fermentum* to hydrolyze lactose was pH – 4.0, temperature – 30°C, lactose concentration – 0.5%, and reaction time – 60 min.

In conclusion, the enzyme β-galactosidase from *L. fermentum* exhibits characteristic features that will enhance its scope of utility in wide industrial applications.

REFERENCES

1. Vasiljevic T, Jelen P. Lactose hydrolysis in milk as affected by neutralizers used for the preparation of crude β-galactosidase extracts from *Lactobacillus bulgaricus* 11842. Innov Food Sci Emerg Technol 2002;3:175-84.
2. Domingues L, Lima N, Teixeira JA. *Aspergillus niger* β-galactosidase production by yeast in a continuous high density reactor. Proc Biochem 2005;40:1151-4.
3. Montanari G, Zambonelli C, Grazia L, Benevelli M, ChiavariC. Release of β-galactosidase from lactobacilli. Food Technol Biotechnol 2000;38:129-33.
4. Voget CE, Flores MV, Faloci MM, Ertola RJ. Effects of the ionic environment on the stability of *Kluyveromyces lactis* β-galactosidase. Lebensm Wiss Technol 1994;27:324-30.
5. Awan SS, Khan SA, Rehman ZU, Saleem A, Rana SM, Rajoka MI. Influence of nitrogen source on the production of β-galactosidase by *Asprergillus niger*. Afr J Biotechnol 2010;9:2918-22.

Source of support: Nil; Conflict of interest: None Declared